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Ammonia toxicity: from head to toe?

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Ammonia toxicity: from head to toe?

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Abstract

Ammonia is diffused and transported across all plasma membranes. This entails that hyperammonemia leads to an increase in ammonia in all organs and tissues. It is known that the toxic ramifications of ammonia primarily touch the brain and cause neurological impairment. However, the deleterious effects of ammonia are not specific to the brain, as the direct effect of increased ammonia (change in pH, membrane potential, metabolism) can occur in any type of cell. Therefore, in the setting of chronic liver disease where multi-organ dysfunction is common, the role of ammonia is challenged. This review provides insights and evidence that increased ammonia can disturb many organ and cell types and hence lead to dysfunction.

Effect of ammonia on cell function

Ammonia is used for a number of different metabolic reactions (including the synthesis of non-essential amino acids). However it is primarily a waste product of cellular metabolism and due to its harmful properties at higher concentrations, ammonia is quickly detoxified and transformed into less toxic compounds (Cooper and Plum, 1987; Butterworth, 2002). Under physiological conditions, blood levels of ammonia (in systemic circulation) are carefully maintained at concentrations $<50\mu\text{M}$ in adults, $50\text{-}75\mu\text{M}$ in term neonates and $50\text{-}150\mu\text{M}$ in preterm neonates (Braissant et al., 2013). Here, the liver plays a vital role in regulating the levels of ammonia via the production of urea (a cycle of enzymes which together are solely found in the liver). Ammonia is also removed in extrahepatic organs, including the muscle and brain (astrocytes), through the amidation of glutamate to glutamine via the enzyme glutamine synthesis (GS) (Cooper and Plum, 1987).

The main source of ammonia generation occurs in the intestines; from lysis of blood-borne urea and also from protein digestion/deamination by urease-positive bacteria and microbial deaminase (Jones et al., 1969). A large amount of metabolically-generated ammonia is absorbed into the blood and via the portal vein is detoxified through the liver (Cooper and Plum, 1987; Brusilow et al., 2010). The concentration of ammonia in portal vein can be 5-10x higher than in the systemic circulation (Abdo, 2006) and during conditions of liver disease or failure, ammonia is poorly removed (due to impaired ureagenesis from hepatocellular dysfunction and portosystemic shunting) and is liberated into the systemic circulation and exposed to all organs (Rovira et al., 2008). Glutaminase, an enzyme which generates glutamate and ammonia from glutamine and which is found in the intestine, kidney and brain (neurons), also plays a contributing role to the development of hyperammonemia. Diseases causing hyperammonemia include acute liver failure (ALF), chronic liver disease (CLD) and portal-systemic shunting (Felipo and Butterworth, 2002). Hyperammonemia also develops in children with inborn errors of the urea cycle and tend to present with higher blood ammonia concentrations (up to 5 mM) compared to acquired causes (such ALF and CLD) which usually result in hyperammonemia levels between 0.2-1 mM (Ratnakumari et al., 1992; Matkowskyj et al., 1999; Butterworth, 2002; Lichter-Konecki et al., 2008).

Ammonia in solution (i.e blood) is present as NH_3 and NH_4^+ with the ratio $\text{NH}_3/\text{NH}_4^+$ depending on the pH as defined by the Henderson-Hasselbach equation. Under physiological conditions with a blood pH of 7.4, more than 98% of ammonia is in NH_4^+ form (Bromberg et al., 1960). Both forms affect pH, electrolytic, acid-base and ion equilibrium. NH_3 is a weak base in gaseous form and uncharged, therefore lipid soluble and capable to cross cell membranes through diffusion. Conversely, NH_4^+ is a weak acid, water soluble and because of its ionic properties crosses cell membranes at a less rapid rate through various transport channels (Bosoi and Rose, 2009). Due to almost identical hydration radius with K^+ (Kikeri et al., 1989), NH_4^+ can substitute K^+ in its channels and transporters (ATPase transporters Na^+/K^+ and H^+/K^+ (Moser, 1987) and $\text{Na}^+/\text{K}^+/\text{Cl}^-$ co-transporters (Kelly et al., 2009)). This NH_4^+ substitution in K^+ channels mediates membrane depolarization *in vitro* (Allert et al., 1998; Norenberg, 1998), but putatively not *in situ* (Rangroo Thrane et al., 2013). Ammonia can also enter cells through aquaporin-8 channel (Liu et al., 2006; Saporov et al., 2007) and ammonia specific transporters (human non-erythroid Rhesus glycoprotein B and C (Bakouh et al., 2006)). There is also growing

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4 evidence that ammonia can cross blood-brain barrier (BBB) preferentially by active
5 transport through ion transporters rather than diffusion (Sørensen, 2013) and it has been
6 shown that ammonia invade paracellular and transcellular passage of different molecules
7 across the BBB (Braissant, 2012; Skowronska and Albrecht, 2012), as well as to a lesser
8 extent by direct diffusion of NH₃. Furthermore, ammonia primarily enters astrocytes lining
9 the BBB as these cells have the highest affinity for potassium (Marcaggi et al., 2004; Bosoi
10 and Rose, 2009; Rangroo Thrane et al., 2013).

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13 Ammonia alters both intracellular and extracellular pH and can cause intracellular
14 acidification as well as alkalinisation, all depending on ammonium concentration, pH and
15 rate of NH₃ vs. NH₄⁺ transport through the cell membrane (Norenberg, 1998; Bosoi and
16 Rose, 2009; Rangroo Thrane et al., 2013). Ammonia has also be shown to cause a rise in
17 intracellular Ca²⁺ primarily due to cytosolic alkalinisation *in vitro* (Rose et al., 2005) and
18 through less well characterized mechanisms lead to increased intracellular Ca²⁺ signalling
19 *in vivo* (Rangroo Thrane et al., 2013). Ammonia-induced changes in pH, membrane
20 potential as well as alterations in cellular metabolism negatively impacts cell function by
21 influencing signalling transduction pathways, activities of many enzymes, alternation in
22 protein phosphorylation and the state of various other ion channels and transporters (Busa
23 and Nuccitelli, 1984; Norenberg, 1998; Cudalbu, 2013). Furthermore, increased ammonia
24 metabolism will lead to metabolic disturbances. NH₄⁺ detoxification by converting α-
25 ketoglutarate to glutamate and glutamate to glutamine leads to α-ketoglutarate depletion
26 consequently stressing the tricarboxylic acid cycle (Braissant et al., 2013).

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29 Elevated concentrations of ammonia have been shown to generate free radicals
30 (Kosenko et al., 1997; Murthy et al., 2001; Sinke et al., 2008; Norenberg et al., 2009) and
31 leads to excessive production of nitric oxide (NO) by stimulating the citrulline-NO cycle
32 (Braissant et al., 1999; Bachmann et al., 2004; Zielinska et al., 2011). Alteration of NO
33 synthesis and oxidative stress can result in the induction of mitochondrial permeability
34 transition (MPT) (Halestrap et al., 1997; Kowaltowski et al., 2001), alterations of BBB
35 permeability (in some disease models) (Rangroo Thrane et al., 2012; Skowronska and
36 Albrecht, 2012; Braissant et al., 2013) and activation of MAPKs (Cagnon and Braissant,
37 2009). Ammonia also activates the transcription factor NF-κB, involved in immune and
38 inflammatory reactions, (Sinke et al., 2008) probably as a result of increased oxidative
39 stress, activated MAPKs and induced MPT (Marchetti et al., 1996; Bowie and O'Neill,
40 2000; Kyriakis and Avruch, 2001).

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43 In addition, liver function impairment independently provokes deviation from
44 physiological values of over 20 different compounds in the circulation (Zieve, 1987) as
45 well as many other factors including inflammation and oxidative stress. In some cases it
46 can be very difficult to distinguish the independent effect of ammonia and therefore other
47 factors (synergistic effects) should be considered (Butterworth, 2008; Bosoi et al., 2012;
48 DeMorrow, 2013; Aldridge et al., 2015).

52 53 **The effects of ammonia toxicity on the brain**

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55 In the brain, ammonia homeostasis is tightly regulated and linked to recycling of
56 the major excitatory and inhibitory neurotransmitters glutamate and γ-aminobutyric acid
57 (GABA) (Paulsen et al., 1987; Bak et al., 2006; Hertz and Kala, 2007). Interestingly, GS
58 in astrocytes has a higher affinity for ammonia than glutamate, indicating that the removal
59 of ammonia may be more important than that of the principle excitatory neurotransmitter
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4 (Waniewski, 1992). Ammonia neurotoxicity is a phenomenon that affects all cerebrate
5 species, from fish to humans (Ip and Chew, 2010). Gross neurological dysfunction occurs
6 in conditions that lead to excess blood and therefore brain ammonia, including
7 encephalopathy, seizures, ataxia and coma (Butterworth, 2002).
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9 Toxic levels of ammonia and alterations in pH, electrolyte disturbances, membrane
10 potential depolarization, are thought to lead to neurological dysfunction primarily by
11 causing cellular swelling accompanied by brain edema and metabolic dysfunction (Bosoi
12 and Rose, 2009). Ammonia is likely to be particularly toxic to astrocytes as they are the
13 only cells that possess the enzyme GS responsible for detoxifying ammonia in the brain
14 through condensation with glutamate (Martinez-Hernandez et al., 1977; Hertz and Zielke,
15 2004). In fact, astrocytes are likely to take up four times more ammonia than any other cell
16 type in the brain (Cooper et al., 1979). Moreover, one of the early pathophysiological
17 findings in HE was the presence of brain edema, and specifically astrocyte swelling in
18 histological specimens and *in vitro*. Astrocyte swelling is also found in the later stages of
19 HE in most animal models of liver failure and hyperammonemia (Gregorios et al., 1985a,
20 1985b; Ganz et al., 1989; Willard-Mack et al., 1996; Tanigami et al., 2005; Cagnon and
21 Braissant, 2007; Jayakumar et al., 2008; Butterworth et al., 2009; Rangroo Thrane et al.,
22 2012; Rao et al., 2014). These findings correlated with increased ammonia and glutamine
23 levels and it was therefore hypothesized that ammonia exerted its neurotoxic effects by
24 accumulation of glutamine causing astrocyte swelling (termed *osmotic gliopathy*).
25 However, astrocyte swelling in the context of ammonia neurotoxicity had until recently not
26 been directly visualized in living tissue. In a hyperacute model of isolated
27 hyperammonemia (urea cycle deficiency), doses of ammonia sufficient to cause stupor,
28 seizures and ataxia did not induce astrocyte swelling *in vivo* (Rangroo Thrane et al., 2013).
29 Additionally, deleting the main astrocyte water channel, aquaporin 4 (AQP4), did not affect
30 disease outcome. However, lethal doses of parenteral ammonia did cause brain swelling *in*
31 *vivo* and astrocyte swelling *in situ* (Rangroo Thrane et al., 2013), and AQP4 deletion does
32 appear to protect against brain edema in the context of HE (Rao et al., 2014). Therefore,
33 although brain edema is a prominent and widely acknowledged clinical feature of the later
34 stages of HE and contributes to mortality, it does not explain all the neurotoxic effects of
35 ammonia (Joshi et al., 2014).
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37 Several alternate and/or synergistic neurotoxic effects of ammonia on astrocytes
38 have previously been studied. These include impairment of oxidative metabolism; with a
39 consequent increase glycolysis (mainly in astrocytes) and dangerously elevated brain
40 lactate levels (Ott et al., 2005; Dam et al., 2013; Bosoi et al., 2014). Ammonia itself is also
41 widely known to cause pH changes that might affect cellular function due to its ability to
42 act both as a weak acid and base. However, *in vivo* studies using liver failure models, pH
43 electrodes and NMR show either an overall increase of intracellular pH ranging from 0.1
44 to 0.4 or no change (Fitzpatrick et al., 1989; Swain et al., 1991; Kanamori and Ross, 1997;
45 Rangroo Thrane et al., 2013). The main limitations of these studies are the indirect
46 measures of pH and the inability to accurately distinguish intra- from extracellular pH.
47 Ammonia has also recently been shown to impair astrocytic calcium signaling *in vivo*,
48 which is closely linked to many astrocytic housekeeping functions, such as K⁺ homeostasis
49 (Rose et al., 2005; Wang et al., 2012; Rangroo Thrane et al., 2013). Additionally, ammonia
50 appears to directly disrupt astrocyte potassium buffering *in vivo* by competing for transport
51 via either the Na⁺-K⁺-ATPase, K⁺ channels or Na⁺-K⁺-Cl⁻ cotransporters (Alger and Nicoll,
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4 1983; Brookes and Turner, 1993; Marcaggi et al., 2004; Rangroo Thrane et al., 2013). An
5 acute increase in both extracellular ammonia and potassium has been shown to cause a
6 depolarizing shift in the GABA equilibrium potential (E_{GABA}), thus leading to decreased
7 neuronal inhibition *in vivo* (Lux, 1971; Benjamin et al., 1978; Rangroo Thrane et al., 2013).
8 This effect likely explains the seizure phenotype seen in models of urea cycle deficiencies
9 (Cagnon and Braissant, 2007). Additionally, ammonia has been found to contribute to
10 seizure generation in epileptic children with normal liver function (Yamamoto et al., 2013),
11 and reactive gliosis seen in temporal lobe epilepsy may induce seizures via an almost
12 identical mechanism (Robel et al., 2015).
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15 More recently, a contribution of other cell types than astrocytes to the
16 pathophysiology of HE and hyperammonemia has gained greater recognition (Butterworth,
17 2011). Microglia are the resident immune cells in the brain, and known to survey the brain
18 microenvironment for signs of pathogens and inflammation with their fine processes
19 (Nimmerjahn, 2005). When activated, they become more amoeboid in structure,
20 facilitating phagocytosis and release multiple pro-inflammatory mediators (Ransohoff and
21 Cardona, 2010). In the late stages of HE, histological studies have shown that microglia
22 are activated in animal models of CLD, ALF, portocaval shunting and in post mortem
23 tissue from patients (Jiang et al., 2009a, 2009b; Rodrigo et al., 2010; Agusti et al., 2011;
24 Zemtsova et al., 2011). Several studies indicate that this microglial activation is correlated
25 with BBB opening and brain edema (Jiang et al., 2009a, 2009b; Rangroo Thrane et al.,
26 2012). However, microglia were not found to be activated in a model of portal vein ligation
27 (Brück et al., 2011) and acute isolated hyperammonemic mice (Rangroo Thrane et al.,
28 2012). It is therefore possible that the microglial activation is not a response to ammonia
29 *per se*, but rather relates to other secondary features of ammonia neurotoxicity such as
30 oxidative stress, cellular distress signals or BBB opening. Finally, ammonia likely has a
31 range of direct and indirect effects on neurons, pericytes and endothelial cells, which have
32 been more extensively reviewed in other publications (Szerb and Butterworth, 1992;
33 Rodrigo and Felipo, 2006; Leke et al., 2011; Thumburu et al., 2012; Shaik et al., 2013).
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39 In summary, the best described deleterious effects of ammonia are associated with
40 the brain as the brain is exquisitely sensitive to even minor increases in the blood ammonia
41 levels. Within the brain, although elevated levels of ammonia are noxious for astrocytes, it
42 also has deleterious effects on all cells of the central nervous system (**Figure 1**). However,
43 can ammonia be considered solely as a neurotoxin? The toxic effects of ammonia are not
44 specific to the brain and there is evidence depicting ammonia toxicity is diffused to
45 organs/tissues and cells in the body.
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48 **The effects of ammonia toxicity on muscle**

49 Sarcopenia or loss of skeletal muscle mass is the most frequent and potentially
50 reversible complication in cirrhosis (Dasarathy, 2012). Despite the universally recognized
51 adverse consequences on survival, development of other complications in cirrhosis, quality
52 of life, and post liver transplant outcomes, there are no effective therapies primarily
53 because the mechanisms of sarcopenia are incompletely understood (Dasarathy, 2012). In
54 addition to the lower muscle mass, muscle strength is also reduced in cirrhosis and
55 contributes to adverse clinical outcomes (Jones et al., 2012). Recent evidence strongly
56 suggests that hyperammonemia is a mediator of the liver muscle axis (Qiu et al., 2013). It
57 is also important to emphasize that identifying ammonia as a mediator of the liver-muscle
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4 axis is especially relevant because effective therapeutic strategies to reverse
5 hyperammonemia are available (Phongsamran et al., 2010).

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7 Previous studies have reported an increased muscle uptake of ammonia in cirrhosis
8 results in glutamine synthesis that is released into the circulation (Ganda and Ruderman,
9 1976; Lockwood et al., 1979; Holecek, 2013). Muscle uptake and metabolism of ammonia
10 is however not a benign process since a number of molecular and metabolic perturbations
11 have been reported during muscle hyperammonemia (Qiu et al., 2012, 2013). The
12 consequences of increased muscle uptake of ammonia include both a reduction in muscle
13 mass and muscle strength (Shanely and Coast, 2002; Qiu et al., 2012). Both metabolic and
14 molecular responses to muscle hyperammonemia contribute to muscle loss and weakness.
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17 *Metabolic perturbations during hyperammonemia*

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19 Since ureagenesis in the liver is impaired and muscle cannot generate urea,
20 ammonia removal by the muscle utilizes a cataplerotic conversion of α -ketoglutarate, a
21 critical tricarboxylic acid cycle intermediate to generate glutamate and glutamine thereby
22 removing 2 moles of ammonia for each mole of α -ketoglutarate. However, the conversion
23 of α -ketoglutarate to glutamate, the first step in this reaction is catalyzed by glutamate
24 dehydrogenase that has a low affinity for ammonia promoting the anaplerotic direction
25 generating α -ketoglutarate from glutamate rather than glutamate from α -ketoglutarate
26 (Wootton, 1983). Under most physiological conditions, since muscle ammonia
27 concentrations do not reach the K_m of this enzyme (~ 1 mM), there is not net cataplerosis
28 of α -ketoglutarate. In cirrhosis, we have consistently reported tissue concentrations of
29 ammonia ~ 4 mM that favors cataplerosis of α -ketoglutarate to glutamate. This is consistent
30 with our observations of lower TCA cycle intermediates in the skeletal muscle during
31 hyperammonemia. Impaired muscle mitochondrial function and ATP generation
32 accompany lower TCA cycle intermediates. These observations provide a mechanistic
33 basis for the previously reported impaired skeletal muscle mitochondrial electron chain
34 complex function in cirrhosis. Reduction in muscle ATP also contributes to lower protein
35 synthesis since peptide chain elongation is a high energy-requiring step. Increased
36 autophagy is a homeostatic response and provides amino acids for anaplerotic reactions.
37 Additionally, reduction in ATP generation also contributes to skeletal muscle contractile
38 dysfunction in cirrhosis.
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44 *Skeletal muscle molecular abnormalities during hyperammonemia*

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46 In addition to skeletal muscle metabolic derangements, ammonia also results in
47 impaired skeletal muscle protein synthesis and increased autophagy. The principal pathway
48 of impaired protein synthesis involves the canonical Akt/mTOR mediated regulation of cap
49 dependent protein synthesis. Myostatin, a TGF- β superfamily member is a potent inhibitor
50 of muscle protein synthesis and inhibits mTOR activation either via an Akt dependent or
51 independent mechanism. Consistently, blocking myostatin increases muscle mass and
52 protein synthesis (Dasarathy et al., 2011).
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55 Hyperammonemia also increases muscle autophagy that in combination with
56 impaired protein synthesis results in loss of muscle mass (Qiu et al., 2012). Both impaired
57 mTOR signaling and activation of AMPK contribute to the increased autophagy. Ammonia
58 also increases protein nitration either directly or by increased generation of reactive oxygen
59 and nitrogen species and autophagy has been shown to contribute to clearance of post
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4 translationally modified muscle proteins (unpublished data). Posttranslational modification
5 of proteins can potentially impair the actomyosin interaction during muscle contraction and
6 result in impaired muscle contractile function.
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8 9 *Therapeutic relevance of muscle hyperammonemia.*

10 The metabolic and molecular data support our belief that hyperammonemia induces
11 a state of anabolic resistance because previous strategies to increase muscle mass by
12 nutritional and hormonal strategies have not been consistently been effective (Tsien et al.,
13 2012). Our studies using an integrated molecular-metabolic approach have shown the
14 interaction between signaling abnormalities and metabolic demands during muscle
15 hyperammonemia lay the foundation for novel mechanistic therapeutic strategies. Our
16 model supports the use of long term ammonia lowering, myostatin antagonism, stimulating
17 muscle mTOR, and providing anaplerotic substrates to reverse the adverse consequences
18 of hyperammonemia. Mitochondrial dysfunction and generation of mitochondrial reactive
19 oxygen species and resultant oxidative stress responses are also potential targets to reverse
20 the adverse skeletal muscle responses of hyperammonemia (unpublished data).
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22 Consistently, myostatin blocking using either follistatin or myostatin knockout in
23 mice have been reported to be effective in reversing hyperammonemia mediated impaired
24 muscle protein synthesis and contractile function (Dasarathy et al., 2011; Qiu et al., 2012,
25 2013). Even though ammonia lowering strategies have been effective in reversing
26 encephalopathy (Phongsamran et al., 2010), there is little data on the impact on the skeletal
27 muscle. One potential reason is that skeletal muscle contractile proteins are long lived and
28 long-term ammonia lowering strategies may be necessary to result in clinically relevant
29 responses.
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31 Interestingly, recent studies have reported that leucine is an anaplerotic substrate
32 generating of α -ketoglutarate (Schachter and Sang, 1997). Additionally, leucine stimulates
33 mTOR directly providing a compelling therapeutic rationale for the use of leucine to
34 reverse muscle hyperammonemia and its consequences. Consistently, human and animal
35 studies in cirrhosis have shown that high doses of leucine have a beneficial effect in
36 reversing impaired skeletal muscle protein synthesis (Tsien et al., 2015). The use of cell
37 permeable esters of α -ketoglutarate also holds promise as a strategy to increase muscle
38 ammonia disposal. Other potential interventions include enhanced non-hepatic ammonia
39 disposal via non-toxic pathways, antioxidants and mitochondrial stabilizers need to be
40 evaluated. On final note, even though hyperammonemia is a mediator of the liver muscle
41 axis, plasma ammonia is elevated in patients with chronic pulmonary disease, severe heart
42 failure and cancers all of which are accompanied by significant skeletal muscle loss
43 (Bessman and Evans, 1955; Chance et al., 1988; Calvert et al., 2010). Therefore, the current
44 approach has broad therapeutic potential across multiple organ dysfunctions.
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46 In conclusion, hyperammonemia occurs in a number of chronic diseases and
47 potentially contributes to sarcopenia and adverse clinical outcomes. Recent advances in
48 our understanding of the molecular and metabolic responses to hyperammonemia in the
49 skeletal muscle provide a mechanistic basis for developing effective therapies (**Figure 2**).
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51 **The effect of ammonia on other organs**

52 *Kidney*

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4 It has been shown in healthy volunteers that following exposure to
5 hyperammonemia, the kidneys take up net ammonia from the systemic circulation (Owen
6 et al., 1961). However, it has been demonstrated in both cirrhotic patients and
7 hyperammonemic rodents, that the kidney becomes a primary source of ammonia (Owen
8 et al., 1961; Olde Damink et al., 2003; Ytrebø et al., 2006). Elevated systemic ammonia
9 concentrations following CLD may directly interact with glomerular cells and contribute
10 to glomerular injury (Ling et al., 1998). Hyperammonemia also plays a crucial role in
11 tubulointestinal fibrosis (Ling et al., 1998). Gordon et al. displayed evidence ammonia has
12 the capacity to promote tubulointestinal injury due to the interaction of ammonia with the
13 third component of complement, a potent stimulus to the production of reactive oxygen
14 species by polymorphonuclear leukocytes and monocytes (Gordon et al., 1985). In this
15 context, Nath et al. demonstrated that hyperammonemia induces the progression of renal
16 injury, not only through complement cascade, but also through the stimulatory effects of
17 ammonia on renal growth (Nath et al., 1991). In fact, oxidative stress can also cause renal
18 ammoniogenesis which may contribute to progression of renal injury (Dan et al., 2008). By
19 contrast, a very recent study by Satpute et al. observed that subacute exposure to
20 ammonium acetate in rats induces renal necrosis and tubular degeneration, which were not
21 correlated with either oxidative or biochemical changes (Satpute et al., 2014). Indeed, it is
22 apparent that exposure of neutrophils to ammonia impair phagocytosis and promotes
23 increased reactive oxygen species generation that results in oxidative stress at infection
24 sites and collateral damage to host tissue (Shawcross et al., 2008).

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26 During ALF, kidneys are capable of continuous ammonia release into the systemic
27 circulation (Ytrebø et al., 2006). A recent study by Cauli et al. demonstrated in rats with
28 acute liver failure that activation of N-methyl D aspartate (NMDA) receptors contribute to
29 hyperammonemia-induced encephalopathy and kidney damage. Moreover, blocking
30 NMDA receptors with MK-801 improved ammonia elimination and glomerular filtration
31 rate and delayed kidney damage (Cauli et al., 2014), confirming the deleterious effects of
32 high ammonia concentration in kidneys. Furthermore, ammonia administered to rats lead
33 to disturbances in renal sodium handling that was preceded by activation of kidney mitogen
34 activated protein kinases/extracellular signal regulated kinases (MAPK/ERKs) signaling
35 pathways, and consequently renal injury (Bento et al., 2005). It has been shown that
36 kidneys perfused with ammonium salts results in cortical and tubular necrosis, with
37 necrotic kidneys demonstrating depressed creatinine clearance (Orvell and Wesson, 1976).
38 Ammonia exposure also inhibits tubular cell proliferation in primary rabbit proximal
39 tubular epithelial cells, therefore affecting cell replication (Rabkin et al., 1993).
40 Collectively, these findings suggest that hyperammonemia does lead to impaired kidney
41 function and sustaining kidney injury.

42 Liver

43 As indicated above, whilst astrocytes are major effectors of brain injury in liver
44 failure, it is of interest that they share many similar markers of activation with hepatic
45 stellate cells (HSC), which are implicated in the development of liver fibrosis and also the
46 increase in intra-hepatic resistance with evolving liver injury (Rockey, 1997). Indeed, HSC
47 contraction is one of the mechanisms that is central to the development of portal
48 hypertension, a serious haemodynamic consequence of cirrhosis. HSC have been shown to
49 possess GS (Bode et al., 1998) and recent data shows that in rodent models of cirrhosis,
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4 high ammonia concentrations are associated with increased HSC contraction and raised
5 portal pressure and conversely, lowering ammonia decreases HSC activation and
6 ameliorates portal hypertension (Jalan et al., 2016).
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8 In addition to the impact on HSC contraction, elevated ammonia has been shown
9 to promote liver injury through up-regulation of toll-like receptor genes, generation of
10 oxidative stress through activation of resident macrophages and neutrophils and activation
11 of NFκB and iNOS. In addition, ammonia promotes increased hepatocyte apoptosis and
12 changes in cell cycle with higher cyclin D1, thereby further promoting liver injury as liver
13 disease evolves (Jia et al., 2014). It is possible that impaired urea cycle metabolism and
14 reduced liver clearance of ammonia primes the liver and other organs to the impact of acute
15 inflammation and further oxidative stress, as occurs in conditions such as acute-on-chronic
16 liver failure, though this assertion requires mechanistic evaluation.
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20 *Lung*

21 Several studies have discussed the detrimental effects of ammonia on lungs
22 following exposure to ammonia gas. Ammonia inhalation also can damage respiratory tract
23 leading to acute lung injury and pulmonary edema, which can ultimately lead to death
24 (Ortiz-Pujols et al., 2014). Furthermore, ammonia inhalation to rats causes increased
25 oxidative stress, decreased antioxidant enzymes and acute lung injury which was
26 attenuated following α-ketoglutarate treatment (Ali et al., 2012). Ammonia induced acute
27 lung injury have also been shown in rabbits associated with an elevated airway pressure
28 and a decrease in PaO₂ (Sjöblom et al., 1999) and has been shown to induce lung fibrosis
29 (Ohnuma-Koyama et al., 2013). Ammonia and enzymatically active urease released from
30 parasitic cells of *Coccidioides posadasii* contribute to host tissue damage and exacerbate
31 the severity of coccidioidal lung infections (Mirbod et al., 2002; Mirbod-Donovan et al.,
32 2005; Wise et al., 2013). In this context, it has been concluded that enzymatically active
33 urease is partly responsible for increased extracellular ammonia concentrations at sites of
34 lung infection, contributing to both localized host tissue damage and exacerbation of the
35 respiratory disease (Lichtenstein et al., 1997).
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41 **The effect of ammonia on vascular function**

42 Chronic hyperammonemia is believed to decrease cGMP formation and thereby
43 secondary signaling for NO, one of the main regulators of tonic vasodilatation (Cauli et al.,
44 2007). Moreover, as previously mentioned, the promotion of reactive oxygen species
45 generation by ammonia gives rise to further endothelial dysfunction by decreasing
46 expression of dimethylarginine dimethylamino hydrolase (DDAH1), a cytosolic enzyme
47 that metabolizes asymmetric dimethylarginine (ADMA), a key endogenous inhibitor of
48 NO. High ADMA levels have previously been shown to be associated with impaired
49 cerebral perfusion during hyperammonemia (Balasubramanian et al., 2012) and also with
50 high intrahepatic resistance in advanced liver disease (Mookerjee et al., 2007). Similarly,
51 under high oxidative stress conditions, there is increased un-coupling of nitric oxide
52 synthase with less NO bioavailability (Xia et al., 1998) and greater nitration of proteins
53 with the generation of peroxynitrite and nitrotyrosine, further promoting organ injury.
54 Furthermore, important cationic transporters such as the y⁺ LAT2 system are also
55 stimulated by high ammonia levels, resulting in arginine efflux from endothelial cells in
56 exchange for glutamine uptake (Zielinska et al., 2011), and thus loss of substrate for nitric
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oxide synthase to generate NO. Thus at multiple levels, ammonia appears to control endothelial function and resistance to vascular flow, whilst also promoting further organ injury. It is perhaps, therefore, not surprising that with progression to advanced cirrhosis and ascites formation and greater ammonia generation, resistive indices for the middle cerebral artery and renal vasculature have been shown to increase, and correlate with severity of hyperdynamic circulatory dysfunction as noted through high renin levels (Guevara et al., 1998).

Conclusion

Increases in ammonia can lead to changes in pH, membrane potential (due to similar properties of NH_4^+ and K^+) and cell metabolism. While ammonia (hyperammonemia) is unequivocally key in the development of HE, the effects of ammonia toxicity are not specific to the brain. It is therefore possible that the deleterious consequences of increased ammonia can impinge on other organs and to date, there is increasing evidence that ammonia is clearly much more than a neuro-toxin. Evidently the brain is more sensitive to ammonia toxicity and recognizing ammonia tolerance/intolerance in other organs merits to be further investigated. Better understanding of how increased ammonia affects various cells and tissues within the body can help lead to better therapeutic strategies for the management of patients with hyperammonemia.

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Figure 1

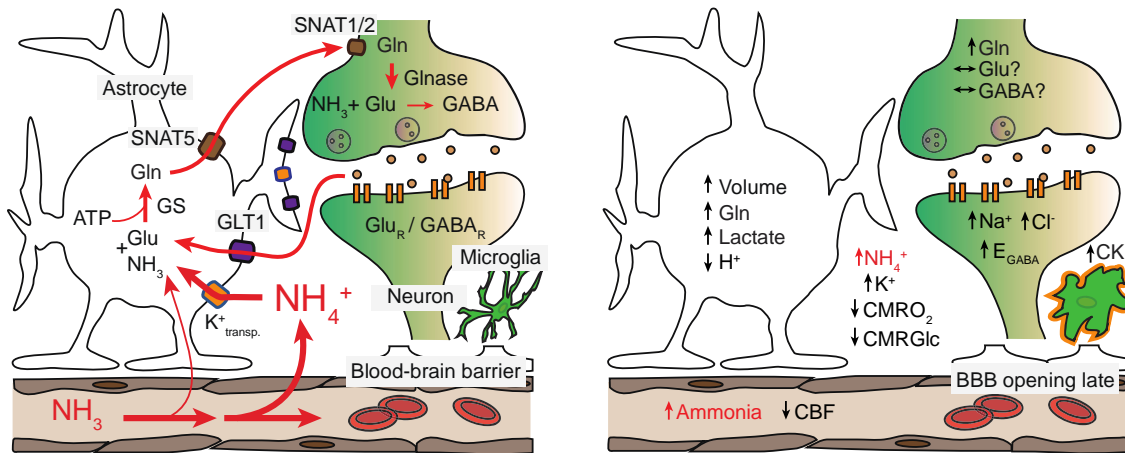


Figure 1. Effects of ammonia toxicity in brain. *Left*, physiological ammonia handling in the brain. *Right*, some of the proposed neurotoxic effects of excess brain ammonia. Glutamate (Glu), glutamine synthetase (GS), glutamine (Gln), adenosine triphosphate (ATP), sodium-coupled neutral amino acid transporters (SNAT1/2 and 5), glutamate transporter 1 (GLT1), glutaminase (Glnase), glutamate and γ -aminobutyric acid (GABA), glutamate receptor (Glu_R), GABA receptor (GABA_R), cerebral blood flow (CBF), cerebral metabolic rate of oxygen (CMRO_2), cerebral metabolic rate of glucose (CMRGlc), GABA reversal potential (E_{GABA}), cytokines (CK).

